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Differential resistance to extended copper exposure in four introduced bryozoans

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ABSTRACT: Resistance to copper by some fouling species may have the 2-fold effect of both facilitating the introduction of nonindigenous species via the painted hulls of vessels, and providing these species with a competitive advantage in ports or estuaries already exposed to anthropogenically elevated copper concentrations. This study tested the tolerance of 4 introduced bryozoans, *Bugula neritina*, *Watersipora subtorquata*, *Schizoporella errata* and *Tricellaria occidentalis*, to a range of Cu concentrations (0, 10, 50, 100 and 500 µg l^{-1}). Larval attachment after 24 h was not found to be a reliable indicator of post-metamorphic survival. Recruits of all species survived in 0 and 10 µg l^{-1} Cu for 20 d, with only *Bugula neritina* and *Watersipora subtorquata* recruits surviving exposure to 50 and 100 µg l^{-1} Cu. *B. neritina* and *W. subtorquata* colonies exhibited reduced post-metamorphic growth in 50 µg l^{-1} Cu compared to controls, with no growth observed in 100 µg l^{-1} Cu. Growth for *S. errata* and *T. occidentalis* was higher at 0 µg l^{-1} than 10 µg l^{-1} Cu. Post-exposure growth of surviving colonies was assessed by transplanting colonies to the field. *W. subtorquata* colonies exposed to 50 µg l^{-1} Cu were the only colonies to show decreased survival and growth post Cu-exposure. Overall, *B. neritina* and *W. subtorquata* showed the greatest tolerance to Cu. These findings have important implications for the management, control and assessment of invasion potential of these invasive marine hull-fouling species.

KEY WORDS: Cu · Attachment · Post-metamorphic survival · Post-metamorphic growth · Antifouling paint · Tolerance · Introduced species · Hull fouling

INTRODUCTION

In many ecosystems the distribution and abundance of organisms is strongly affected by human disturbances. Two of the greatest threats to marine biodiversity and system health worldwide are the spread (or invasion) of introduced marine species (Carlton & Geller 1993, Holloway & Keough 2002, Hayes et al. 2004) and exposure to anthropogenic pollution (Rygg 1985a, Rygg 1985b, Luoma & Phillips 1988, Cohen & Carlton 1998, Preston & Shackelford 2002).

Sessile marine invertebrates in bays and estuaries are among the most vulnerable marine communities to invasion, due to both an abundant supply of invasive propagules (or larvae) through a wide range of transport vectors (Carlton & Geller 1993), and the high levels of anthropogenic disturbance that occur in these locations (Cohen & Carlton 1998). Anthropogenic disturbances can act to weaken and alter the structure of marine invertebrate communities, allowing introduced species to gain a foot-hold and potentially spread (Clark & Johnston 2005).

Copper is a common toxicant in the marine environment that has the potential to affect both the spread of nonindigenous species and the health of epifaunal assemblages in bays and estuaries. Many ports and harbours are exposed to copper pollution via anthropogenic discharge into estuaries, embayments and streams in the form of industrial waste (Apte & Day 1998, Hall Jr. et al. 1998), urban runoff and sewage discharge (Scanes 1996, Pitt 2002), wood preservatives (Weis & Weis 1992, 1996) and from boat hulls via antifouling biocides (Paulson et al. 1989). While copper is a trace element found naturally at low concentrations in the aquatic environment, at higher concentrations it is considered one of the 3 most toxic heavy metals to

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marine invertebrates of several trophic groups (Hall et al. 1998). Copper has been shown to decrease the reproductive success, growth rates and abundance of many species (Hall et al. 1998), and lead to changes in the structural composition of benthic communities (Weis & Weis 1992, Johnston et al. 2002).

In recent years, hull fouling of ships and recreational vessels has been identified as an important vector for the transport and spread of nonindigenous species (Minchin & Gollasch 2003, Floerl et al. 2004, Hewitt et al. 2004). The frequency of nonindigenous species transport may increase as tributyl tin (TBT), the primary antifouling biocide used on most large commercial vessels worldwide, is phased out by 2008 (I.M.O. 2001), and replaced by alternative less effective biocides such as copper. Floerl et al. (2004) found that despite the use of copper-based antifouling paints on boat hulls, several recognized nonindigenous species were able to colonise the primary and/or secondary surfaces of some vessel hulls.

Thus the possibility exists that resistance to copper by some fouling species may have the 2-fold effect of both facilitating the introduction of nonindigenous species via the hulls of vessels, and providing these species with a competitive advantage in ports or estuaries already exposed to anthropogenically elevated copper concentrations.

As such there exists a need to quantify the extent to which known nonindigenous ship hull fouling species are able to establish and grow in the presence of a copper toxicant. While many studies exist on the toxicity of copper to invertebrate species, they are generally concerned with determining LC50's or dose-response relationships in the laboratory (Abel 1989, Callow 1994). Larvae are generally recognised as the invertebrate life-stage most sensitive to toxicants (Connor 1972, Calabrese et al. 1973, McKim 1977) and as a result studies into the effect of toxicants on invertebrates often end shortly after larval metamorphosis. The duration of such studies is generally <96 h, with little concern for the effects of prolonged exposure (several weeks) or the recovery success of organisms once exposure has ceased (but for exceptions see Ng & Keough 2003). While this approach is beneficial for testing the short-term events such as attachment or settlement, it may be of limited use to the overall management of nonindigenous species, since the survival and growth of only a few individuals from a population may be sufficient for a successful invasion to occur.

The aims of this study were to investigate and compare the toxicity of copper to 4 cosmopolitan bryozoan species, *Bugula neritina*, *Watersipora subtorquata*, *Schizoporella errata* and *Tricellaria occidentalis*. Bryozoans form an abundant component of most epibenthic communities (Gordon & Mawatari 1992), and are common fouling organisms on the hulls of ocean-going vessels (Gordon & Mawatari 1992, Minchin & Gollasch 2003, Floerl et al. 2004). All 4 bryozoans species in this study are recognised as national priority pest species within Australian waters, with invasion potentials ranging from medium to high (Hayes et al. 2004). Studies have already demonstrated that *B. neritina* and *W. subtorquata* have the ability to grow on (Floerl et al. 2004) and around (Johnston & Webb 2000) surfaces treated with copper-based antifouling paints, however little research exists on the settlement, survival and growth success of *S. errata* and *T. occidentalis* in the presence of copper. The specific aims will be to: (1) determine the attachment success of bryozoan larvae and (2) the post-metamorphic survival and growth of colonies exposed to a range of copper concentrations over 20 d, and (3) assess the post-exposure survival and growth of field-transplanted colonies (up to 4 wk).

This study sets a benchmark by which to compare the tolerance of endemic species to copper so that we may better understand the role of toxicants in facilitating the introduction of nonindigenous species into native assemblages.

MATERIALS AND METHODS

Sample collection. Adult colonies of *Bugula neritina*, *Watersipora subtorquata* and *Tricellaria occidentalis* were collected from Caltex Pier, Kurnell, in Botany Bay, NSW Australia. Colonies of *Schizoporella errata* were collected from Rose Bay, in Port Jackson, NSW Australia. Colonies were transported and stored in aerated tanks of filtered seawater collected from their respective field sites. They were maintained in darkness for 2 d before being induced to spawn through exposure to bright light (Wisely 1958).

Copper treatments. In all experiments, analytical grade copper II chloride hydrous ($CuCl_2.2H_2O$) was used as the reference toxicant. A 1000 mg l^{-1} Cu stock solution was prepared by dissolving 1.34 g of $CuCl_2 \cdot 2H_2O$ in 500 ml of Milli-Q® filtered water. Stock solution was stored at 4°C to prevent reduction of Cu ions in solution. A 1000 µg l^{-1} Cu solution was prepared from this stock solution each day and diluted in order to obtain all experimental treatment solutions of 10, 50, 100 and 500 µg l^{-1} Cu. Filtered seawater collected from the field sites was used as the dilution medium. Seawater was filtered through a 0.2 µm filter to reduce the possibility of complexation of Cu with organic particles and maximise the amount of biologically available Cu in solution (Campbell 1995, Batley et al. 2004). All equipment was acid washed in 5% nitric acid for a minimum of 24 h and rinsed in Milli-Q® filtered water prior to use.

In preparation for analysis, 60 ml sub-samples of Cu treatment solutions were collected at the commencement of the experiments and immediately acidified with analytical grade nitric acid (1.5 µl of acid per 1 ml of sample) and refrigerated. The actual concentration of copper in stock and experimental solutions was then independently tested by the Australian Government National Measurement Institute (detection limit of 5 µg l^{-1}).

20 d toxicity tests. The attachment success and post-metamorphic survival and growth of bryozoan larvae and recruits exposed to copper were determined during 20 d laboratory toxicity experiments conducted in April and May 2004. Plastic Petri dishes (35 mm in diameter) were used as the experimental containers. Each dish was acid washed then pre-soaked in the appropriate copper solution for 24 h prior to commencing the experiments to ensure minimal chelation (or binding) of any bioavailable Cu in solution during the exposure period. Pilot studies indicated that larvae of some bryozoan species in this experiment develop to fully feeding settled individuals between 1 and 2 d after attachment and metamorphosis. Therefore, from Day 2 onwards a food source (the microalgae *Isochrysis galbana*) was included as a component of each treatment solution at a concentration of 10^5 cells ml^{-1}. Algal sorption has the potential to reduce the bioavailability of Cu by up to 20% in solutions with high Cu concentrations (e.g. 50, 100 and 500 µg l^{-1}) and up to 45% in solutions of low Cu concentration (e.g. 10 µg l^{-1}) (as shown in Xie et al. 2005). Due to this, nominal copper concentrations in this study are effectively range values, with the concentrations used in each experiment being 0, 5–10, 40–50, 80–100 and 400–500 µg l^{-1} Cu, with 6 replicate dishes for each treatment. For ease of presentation of results however, these nominal concentration ranges will continue to be referred to as 0, 10, 50, 100 and 500 µg l^{-1} Cu. Concentrations ranges of 5–10 and 40–50 µg l^{-1} Cu represent relevant values that do exist in polluted aquatic environments (Stauber et al. 2000, Schiff et al. 2004), while 80–100 and 400–500 µg l^{-1} Cu concentrations were primarily included to gauge maximum tolerance limits. Exposure to treatments was maintained for 20 d. Solutions were replaced daily with solutions prepared from stock solution immediately prior to use.

In order to reduce the size of the experiment, treatment dishes were inoculated with the larvae of 2 bryozoan species. One series of treatment dishes was inoculated with larvae of *Bugula neritina* and *Schizoporella errata*, while a second treatment series was inoculated with *Watersipora subtorquata* and *Tricellaria occidentalis* larvae. The total volume of treatment solution in each dish was 4.5 ml. At no time during the experiments did the organism loading come close to exceeding the specified ATSM 1192-97 guidelines of 0.5 to 0.8 g organism l^{-1} (ASTM 1999), nor was any intra-specific interaction between larvae observed. The number of larvae of each species put into each Petri dish was dependant on the individual spawning success of adult colonies, with 20 larvae of *B. neritina*, 10 larvae of *W. subtorquata* and *T. occidentalis* and 5 *S. errata* larvae being added to each container. Following inoculation of larvae, all treatments were kept in darkness for 24 h to encourage settlement, after which time they were subjected to a shaded 12:12 light:dark cycle. All experiments were conducted at a constant temperature of 20°C.

After 24 h the number of attached larvae was recorded in each container using a dissecting microscope. Attachment was defined as a larva that had attached to the surface of the container and initiated metamorphosis into the ancestrula. The location of individual larvae within the container was mapped for reference. The subsequent survival and growth of settled larvae was recorded at 5, 10, 15 and 20 d. Survival was defined as successful metamorphosis resulting in a zooid with a primary orifice present. A zooid was deemed to be dead if it appeared empty or if only a brown-body (Brusca & Brusca 1990) was visible. Growth was recorded by counting the number of new zooids budded from each ancestrula, with a bud being counted as a new zooid once a primary orifice had developed.

Laboratory-to-field transfer. To assess the recovery ability and possible carry-over effects of copper exposure on the survival and growth of bryozoan colonies all Petri dishes were transferred into the field immediately after completion of the laboratory exposure period (20 d). The laboratory-to-field transfers were conducted between May and June. The field site was located at Caltex Pier, at Kurnell in Botany Bay, NSW Australia, 15 km south of Sydney. The pier extends 1.3 km from the southern shore of the bay, and experiments were deployed approximately 500 m from the shore. Naturally occurring sessile assemblages of sponges, ascidians, bryozoans, polychaetes, hydrozoans, anthozoans and macroalgae have been previously documented at Caltex Pier (Pollard & Pethebridge 2002). The background concentrations of total copper in the water at the pier are less than 5 µg l^{-1} (R. F. Piola & E. L. Johnston unpubl. data).

Petri dishes were randomly arranged onto a PVC backing plate (50 × 50 × 0.5 cm) and attached using silicon glue. The backing plate was suspended in the water column at a depth of 2 m below low water mark with treatment dishes on the underside to minimise available light and sedimentation. A counter-weight was attached below the backing plate to provide stability. Dishes were retrieved from the field after 26 d and transported back to the laboratory and maintained in a recirculating seawater system prior to census.

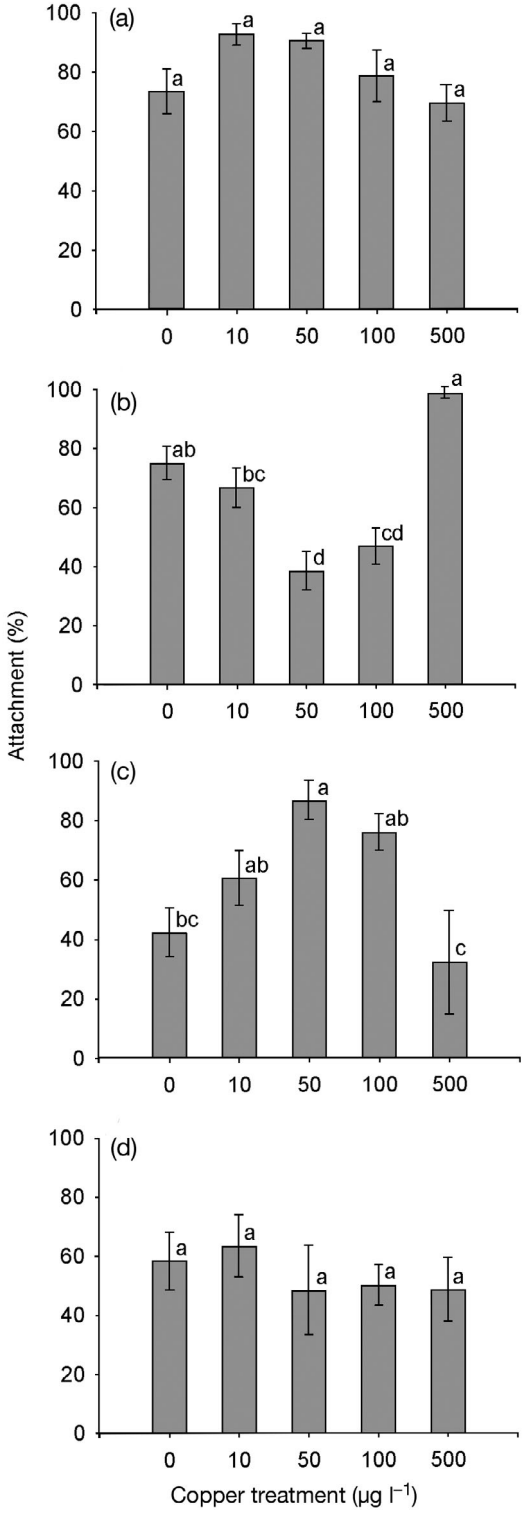

Fig. 1. Effects of 0, 10, 50, 100 and 500 µg l^{-1} Cu treatment on the attachment of (a) *Bugula neritina*, (b) *Watersipora subtorquata*, (c) *Schizoporella errata* and (d) *Tricellaria occidentalis* recruits after 24 h exposure. Bars represent mean (±1 SE). Different letters represent significant differences in Tukey's post-hoc comparisons ($\alpha = 0.05$)

During counting, colonies derived from experimental larvae were distinguished from colonies growing from field recruitment using the mapped positions recorded during the laboratory experiments. The number of colonies surviving in each dish was counted and recorded as a percentage of the colonies present in the dish at the time of field deployment. Due to the differing growth morphologies among the species (*Bugula neritina* and *Tricellaria occidentalis* are arborescent bryozoans while *Watersipora subtorquata* and *Schizoporella errata* are encrusting), 2 different techniques for estimating growth were employed. For the arborescent species, growth was assessed by counting the number of bifurcations along 3 separate branch lengths within any one colony. This number was then averaged to get a mean value for that colony. Bifurcations have been found to be a reliable estimate of colony size (Keough & Chernoff 1987). For the encrusting species, the total number of zooids in each colony was counted with the aid of image analysis software.

Data analysis. Differences in the percent attachment of larvae of each species were analysed using a 1-factor ANOVA with copper treatment as a fixed factor. Tukey's post-hoc tests were conducted on significant results to test for differences between copper treatments. Repeated measures analysis of variance was used to test for the effects of copper on the percent survival and growth of larvae during the 20 d laboratory exposure, with Cu treatment being the between-subjects effect and time the repeated effect. Planned comparisons were carried out on significant results to determine differences between specific Cu treatments, with all planned comparisons tested against the error term for the main test of copper treatment (Quinn & Keough 2002). For the laboratory-to-field transfer a 1-factor ANOVA was used to test for variation in the post-metamorphic survival and growth of recruits with Cu treatment as a fixed factor. Tukey's post-hoc tests were performed on significant results to test for differences between Cu treatments. All data was assessed for homogeneity of variance and normality using plots of residuals versus means and descriptive statistics; and when required analysis was performed on square-root transformed data.

RESULTS

Larval attachment

The proportion of larvae attaching to Petri dishes after 24 h across all Cu treatments and species ranged between 38 and 98% attachment (Fig. 1). There was no significant difference in the attachment rate of *Bugula neritina* ($F_{4,25} = 2.755$, $p = 0.050$; Fig. 1a) or *Tricellaria*

occidentalis ($F_{4,25}$ = 0.382, p = 0.819; Fig. 1d) larvae across Cu treatments, though *T. occidentalis* showed decreased attachment overall (48 to 63%) compared with *B. neritina* (73 to 93%; Fig. 1a,d). Attachment of *Watersipora subtorquata* larvae varied between treatments ($F_{4,25}$ = 17.726, p < 0.001; Fig. 1b) with reduced recruitment at 50 and 100 µg l^{-1} Cu compared to the control and 10 µg l^{-1} Cu treatments (Fig. 1b). Interestingly there was almost 98% attachment occurring at the highest Cu concentration of 500 µg l^{-1} (Fig. 1b). Significant differences occurred in *Schizoporella errata* attachment between treatments ($F_{4,24}$ = 6.501, p = 0.001; Fig. 1c), with attachment of larvae increasing from 42% in the control treatment, up to a peak of 87 and 76% in 50 and 100 µg l^{-1} Cu treatments, followed by a minimum of 32% at 500 µg l^{-1} Cu (Fig. 1c).

Post-metamorphic survival and growth

Survival and growth of recruits was assessed from Days 5 to 20 because attachment and the initiation of metamorphosis after 24 h cannot be equated with survival and the completion of metamorphosis into an adult zooid. Census at Day 5 revealed 100% mortality of all bryozoans in the 500 µg l^{-1} Cu treatment (Fig. 2). 100% mortality was also recorded for *Schizoporella errata* and *Tricellaria occidentalis* in 50 and 100 µg l^{-1} Cu treatments (Fig. 2c,d). Overall post-metamorphic survival of recruits was highest in 0 and 10 µg l^{-1} Cu for all species, with only *Bugula neritina* and *Watersipora subtorquata* recruits surviving at 50 and 100 µg l^{-1} Cu (Fig. 2). Where 100% mortality was recorded for a particular Cu concentration at Day 5, this treatment was excluded from the repeated measures analysis as no further potential for a change in survival through time between Days 5 and 20 was possible. Similarly, treatments with insufficient replicates (n < 4) as a result of mortality were also excluded from repeated measures analysis.

There was generally little change in recruit survival between Days 5 and 20 (Fig. 2). There was a significant Cu treatment by time interaction for *Bugula neritina* (Table 1) due to the large mortality of recruits in the 50 µg l^{-1} Cu treatment between Days 5 and 10 (Fig. 2a). Analysis of *Watersipora subtorquata* survival showed a significant difference between Cu treatments (Table 1, Fig. 2b) with planned comparisons showing this difference to exist between 0 and 50 µg l^{-1} Cu from Days 5 to 20 (Table 1, Fig. 2b). Five percent survival was recorded for *W. subtorquata* recruits in 100 µg l^{-1} Cu (Fig. 2b), though this was not included in the analysis. There were no significant differences between 0 and 10 µg l^{-1} Cu treatments for *Schizoporella errata*, with time not used as a factor for analysis since there was no

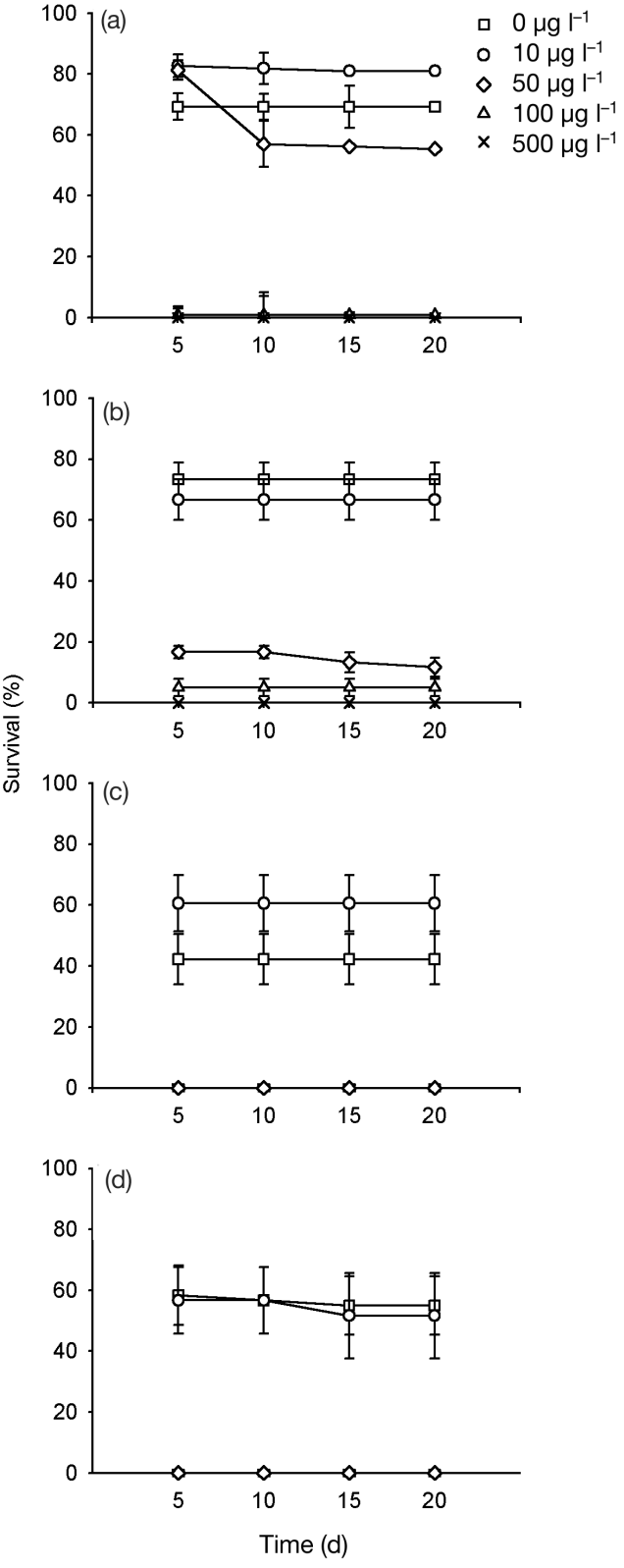

Fig. 2. Effects of 0, 10, 50, 100 and 500 µg l^{-1} Cu exposure on the survival of (a) *Bugula neritina*, (b) *Watersipora subtorquata*, (c) *Schizoporella errata* and (d) *Tricellaria occidentalis* recruits over 20 d. Values represent the mean (±1 SE)

Table 1. Summary of repeated measures analyses for laboratory toxicity experiments on the survival of bryozoan recruits exposed to copper treatments over 20 d. Degrees of freedom (df), mean square errors (MS) and probability (p) values are presented to enable reconstruction of the full analysis table. p-values in bold indicate significant differences at $\alpha = 0.050$; p = 0.000 denotes values <0.0005. –: no analysis conducted due to complete absence of variation in survival over time

Factors	Source	Cu treatments analysed (µg l^{-1})	Main test df	MS	p
Bugula neritina					
Between	Cu treatment	0, 10, 50	2	0.227	0.176
	Error		15	0.116	
Within	Time		3	0.035	**0.003**[a]
	Time × Cu treatment		6	0.030	**0.002**[a]
	Error		45	0.003	
Watersipora subtorquata					
Between	Cu treatment	0, 10, 50	2	2.483	**0.000**
	Error		15	0.064	
Within	Time		3	0.001	0.234[a]
	Time × Cu treatment		6	0.001	0.237[a]
	Error		45	0.001	
Schizoporella errata					
Between	Cu treatment	0, 10	1	0.101	0.168
	Error		10	0.046	
Within	Time		–	–	–
	Time × Cu treatment		–	–	–
	Error				
Tricellaria errata					
Between	Cu treatment	0, 10	1	0.005	0.898
	Error		10	0.299	
Within	Time		3	0.006	0.189[a]
	Time × Cu treatment		3	0.001	0.642[a]
	Error		30	0.003	

[a]Denotes use of Greenhouse-Geisser epsilon adjusted p-values due to assumption of sphericity violation (when G-G epsilon <0.7)

change in survival for either of the treatments for the duration of the experiment (Table 1, Fig. 2c). No significant difference was recorded between 0 and 10 µg l^{-1} Cu treatments or time for *Tricellaria occidentalis* (Table 1, Fig. 2d).

Post-metamorphic growth was assessed as the change in the average number of live zooids per colony. As for survival analysis, growth was analysed only for treatments with <100% mortality at Day 5, with the exception of *Bugula neritina* and *Watersipora subtorquata* at 100 µg l^{-1} Cu which were omitted from the analyses because survival was recorded in only 1 and 2 replicates respectively. Therefore the Cu treatments analysed for growth differed among species, with 0, 10 and 50 µg l^{-1} Cu analysed for B*. neritina* and *W. subtorquata*, and 0 and 10 µg l^{-1} Cu for *Schizoporella errata* and *Tricellaria occidentalis* (Table 2).

A significant Cu treatment by time interaction was observed in post-metamorphic growth for all species (Table 2). The planned comparisons for *Bugula neritina* and *Watersipora subtorquata* showed that copper concentrations of 50 µg l^{-1} Cu reduced growth compared to controls at Days 15 and 20 (Table 2, Fig. 3a,b). Some growth (addition of new zooids) was observed among surviving colonies of both *B. neritina* and *W. subtorquata* at 50 µg l^{-1} Cu (Fig. 3a,b), however for *B. neritina* the average number of live zooids per colony remained essentially unchanged after 10 d due to concurrent mortality of older zooids within the colonies (Fig. 3a). There was no growth observed at 100 µg l^{-1} Cu for either of these species (Fig. 3a,b). Growth for *Schizoporella errata* was much higher at 0 than 10 µg l^{-1} Cu by Days 15 and 20 (Table 2, Fig. 3c). The highest growth among all species at both 0 and 10 µg l^{-1} Cu was recorded for *T. occidentalis* (Fig. 3d), with a significant Cu treatment by time interaction occurring (Table 2). Planned comparisons showed this difference to be significant at Day 20 ($F_{1,10} = 5.006$; p = 0.049).

The nominal and measured Cu concentrations taken at the commencement of the 20 d larval toxicity experiments are shown in Table 3.

Laboratory-to-field transfer

Survival rates in the field were calculated as a proportion of the number of colonies transplanted alive (not the number of initial inoculants). After 26 d in the field there was some mortality (ranging from 12 to 65%) but this was independent of previous Cu exposure (Fig. 4).

Growth of *Bugula neritina* colonies in the field were not affected by previous copper treatment, with the average number of bifurcations differing by only ~0.1 between the 0 and 50 µg l^{-1} Cu treatments (Table 4, Fig. 5a). *Watersipora subtorquata* growth was dramatically reduced by previous exposure to 50 µg l^{-1} Cu treatments ($F_{3,20} = 36.467$; Table 4, Fig. 5b). Surprisingly, growth was recorded for 2 *W. subtorquata* recruits initially exposed to 100 µg l^{-1} Cu that appeared blackened and dead prior to field deployment (Figs. 5c & 6); however, lack of sufficient replicates for this treatment prevented it from being used in the analysis. There was no difference in *Schizoporella errata* and *Tricellaria occidentalis* growth between 0 and 10 µg l^{-1} Cu, though there was a slight trend in both species for decreased growth with increased copper (Table 4, Fig. 5c,d).

Table 2. Summary of repeated measures analyses for laboratory toxicity experiments on the growth of bryozoan recruits exposed to Cu treatments over 20 d. If there was a significant interaction between copper treatment and time in the main test then planned comparisons were carried out for each copper treatment against the control at each time (Days 5, 10, 15, 20). All planned comparisons were tested against the error term for the main test of Cu treatment. p values in bold indicate significant differences at α = 0.050. p = 0.000 denotes values <0.0005. *: no planned comparisons conducted due to no variation; –: no planned comparisons conducted because Cu treatment omitted from analysis

Factors	Cu	Main test				Planned comparisons											
						Day 5				Day 10				Day 15				Day 20			
Source	treatment analysed	df	MS	p	df	0 vs. 10		0 vs. 50		0 vs. 10		0 vs. 50		0 vs. 10		0 vs. 50		0 vs. 10		0 vs. 50	
						MS	p	MS	p	MS	p	MS	p	MS	p	MS	p	MS	p	MS	p
Bugula neritina																					
Between																					
Cu treatment	0, 10, 50	2	0.709	**0.031**																	
Error		15	0.160																		
Within																					
Time		3	1.777	**0.000**[a]																	
Time × Cu treatment		6	0.195	**0.003**[a]	1, 15	*	*	*	*	0.283	0.203	0.100	0.442	0.183	0.302	1.238	**0.014**	0.024	0.704	0.903	**0.031**
Error		45	0.034																		
Watersipora subtorquata																					
Between																					
Cu treatment	0, 10, 50	2	0.863	**0.001**																	
Error		15	0.076																		
Within																					
Time		3	1.201	**0.000**[a]																	
Time × Cu treatment		6	0.172	**0.002**[a]	1, 15	*	*	*	*	0.042	0.469	0.117	0.234	0.144	0.189	1.238	**0.001**	0.133	0.206	1.303	**0.001**
Error		45	0.029																		
Schizoporella errata																					
Between																					
Cu treatment	0, 10	1	34.082	**0.000**																	
Error		10	0.163																		
Within																					
Time		3	2.757	**0.000**[a]																	
Time × Cu treatment		3	0.641	**0.003**[a]	0, 10	*	*	*	*	0.593	0.076	–	–	3.485	**0.000**	–	–	2.028	**0.003**	–	–
Error		30	0.082																		
Tricellaria errata																					
Between																					
Cu treatment	0, 10	1	82.496	**0.000**																	
Error		10	1.333																		
Within																					
Time		3	8.966	**0.000**[a]																	
Time × Cu treatment		3	2.419	**0.010**[a]	0, 10	0.580	0.524	–	–	0.128	0.763	–	–	1.824	0.269	–	–	6.673	**0.049**	–	–
Error		30	0.298																		

[a]Denotes use of Greenhouse-Geisser epsilon adjusted p-values due to assumption of sphericity violation (when G-G epsilon <0.7)

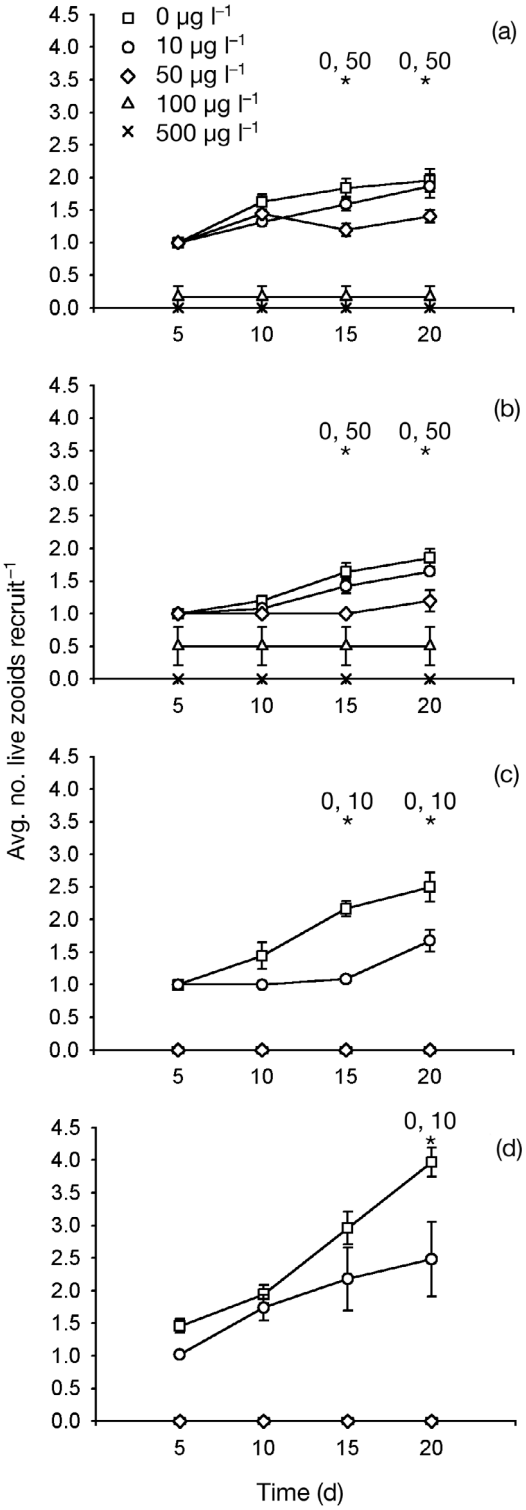

Fig. 3. Effects of 0, 10, 50, 100 and 500 µg l^{-1} Cu exposure on the growth of (a) *Bugula neritina*, (b) *Watersipora subtorquata*, (c) *Schizoporella errata* and (d) *Tricellaria occidentalis* recruits over 20 d. Values represent the mean (±1 SE). *Represent significant differences based on planned comparisons, with numbers shown being the Cu concentrations that differed

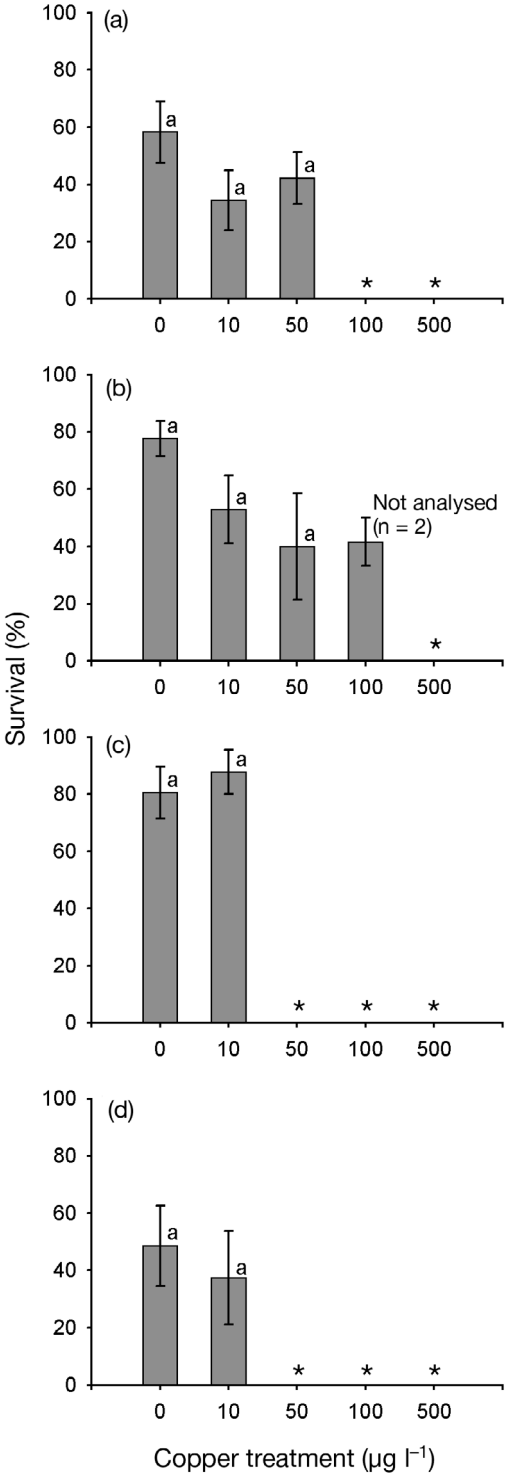

Fig. 4. Survival in the field of (a) *Bugula neritina*, (b) *Watersipora subtorquata*, (c) *Schizoporella errata* and (d) *Tricellaria occidentalis* recruits following 20 d exposure to 0, 10, 50, 100 and 500 µg l^{-1} Cu. Values represent the mean (±1 SE). *Represent Cu treatments that were not transferred to the field due to 0% survival in laboratory trials. Different letters represent significant differences in Tukey's post-hoc comparisons ($\alpha = 0.05$)

Table 3. Nominal and measured Cu concentrations (μg l^{-1}) for Cu treatments used during 20 d toxicity experiments (±1 SE; n = 2). Lowest detection limit for analysis was 5 μg l^{-1}

Nominal concentration	Measured concentration
0	<5
10	12.2 ± 3.9
50	47.5 ± 8.5
100	88.0 ± 12
500	450.0 ± 60

DISCUSSION

The introduced bryozoans *Bugula neritina*, *Watersipora subtorquata*, *Schizoporella errata* and *Tricellaria occidentalis* displayed a wide range of Cu tolerances, and the magnitude of tolerance could not be determined by assessing initial larval attachment. Assessment of the post-metamorphic survival and growth of individuals exposed to Cu over extended time periods (weeks) followed by a period of field transplantation allowed us to determine tolerance, carry-over effects and recovery ability in a real world ecological context.

Larval attachment success after 24 h was the first toxicity test end-point we examined, however this proved an unreliable indicator of successful metamorphosis or survival beyond 24 h. Previous studies have suggested that Cu may induce and accelerate the attachment of *Watersipora* sp. and *Bugula neritina* larvae (Wisely 1962a, b, Ng & Keough 2003). In contrast, our study showed reduced attachment success in *Watersipora subtorquata* up until 500 μg l^{-1} Cu. Moreover, there was no inducement of attachment by Cu in *Bugula neritina* and *Tricellaria occidentalis*, and only a slight increase in *Schizoporella errata* attachment at 50 μg l^{-1} Cu. In contrast to initial attachment success, survival at Day 5 was a substantially better indicator of Cu tolerance. Larvae that were able to attach, metamorphose and survive to Day 5 invariably remained alive for the 20 d duration of the laboratory experiment.

Post-metamorphic survival and growth observations indicated a wide range of Cu tolerance among the introduced bryozoans. While *Schizoporella errata* and *Tricellaria occidentalis* suffered increased mortality and reduced growth following only small increases in Cu (10 μg l^{-1}), *Bugula neritina* and *Watersipora subtorquata* were able to survive in all Cu treatments up to and including 100 μg l^{-1}. One source of Cu in ports and harbours is antifouling paints. *In situ* studies on the release of Cu from freshly painted antifouling coatings show that release rates, while initially high at 25 to 65 μg Cu cm^{-2} d^{-1}, quickly decreased to much lower levels of 9 to 25 μg Cu cm^{-2} d^{-1} after only 30 d of submersion (Valkirs et al. 2003). Since results of this study indicate that larvae of *Bugula neritina* and *Watersipora subtorquata* are able to successfully settle, survive, and grow in Cu concentration well within even this initial range, it is possible that larvae of these species would be able to recruit onto hulls of vessels with freshly painted antifouling coatings. Indeed, field experiments have shown that *W. subtorquata* and *B. neritina* are able to grow directly upon or within close proximity to surfaces coated with copper-based antifouling paints (Johnston & Webb 2000, Floerl et al. 2004). Provided these recruits were then able to survive the initially high Cu concentrations for the first 20 to 30 d post-attachment (as is also demonstrated in this study) then they would have a very good chance of establishing a colony and growing through to reproductive maturity. Combining this scenario with the fact that modern ves-

Table 4. ANOVA for the survival and growth of bryozoan recruits transferred to the field. Recruits had previously been exposed to 0, 10 and 50 μg l^{-1} Cu in the lab. Degrees of freedom (df), mean square errors (MS) and probability (p) values are presented to enable reconstruction of the full analysis table. p-values in bold indicate significant differences at α = 0.050; p = 0.000 denotes values <0.0005

Source	Cu treatments analysed	Survival			Growth		
		df	MS	p	df	MS	p
Bugula neritina							
Cu treatment	0, 10, 50	2	0.087	0.318	2	0.016	0.969
Error		15	0.07		15	0.497	
Watersipora subtorquata							
Cu treatment	0, 10, 50	2	0.205	0.136	2	239.222	**0.000**[a]
Error		14	0.089		14	7.824	
Schizoporella errata							
Cu treatment	0, 10	1	0.016	0.558	1	20.574	0.504[a]
Error		10	0.043		10	42.866	
Tricellaria occidentalis							
Cu treatment	0, 10	1	0.034	0.614	1	14.407	0.330
Error		9	0.125		9	13.616	

[a]Square-root transformed

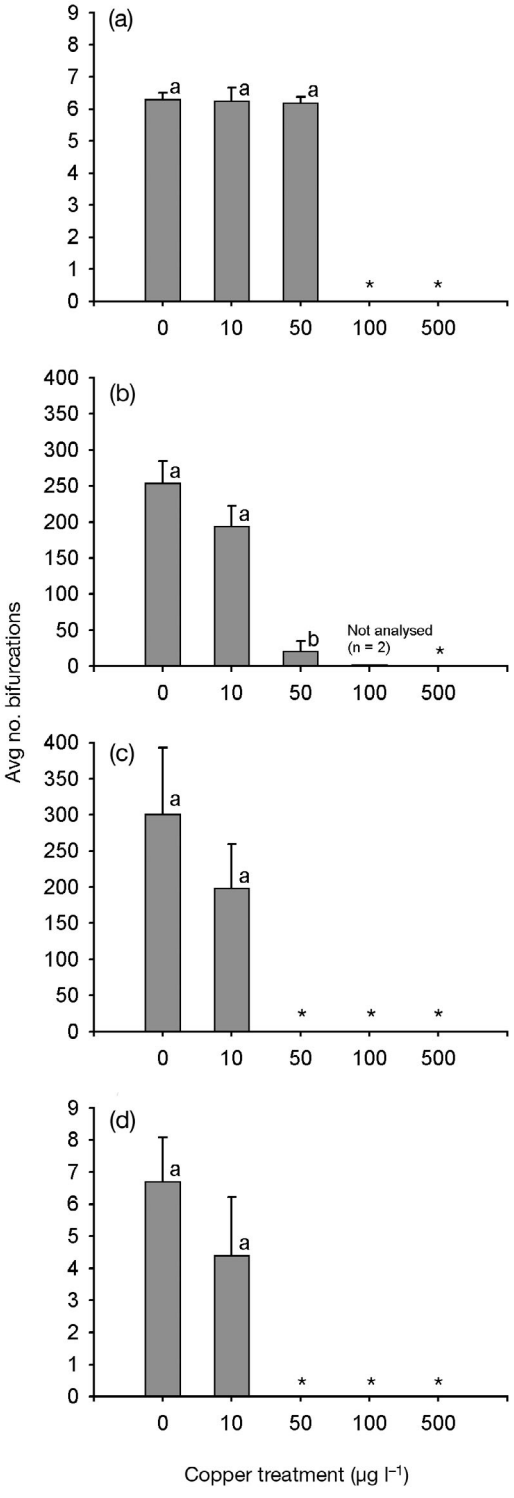

Fig. 5. Growth in the field of (a) *Bugula neritina*, (b) *Watersipora subtorquata*, (c) *Schizoporella errata* and (d) *Tricellaria occidentalis* recruits following 20 d exposure to 0, 10, 50, 100 and 500 µg l^{-1} Cu. Values represent the mean (±1 SE). *Represent Cu treatments that were not transferred to the field due to 0% survival in laboratory trials. Different letters represent significant differences in Tukey's post-hoc comparisons ($\alpha = 0.05$)

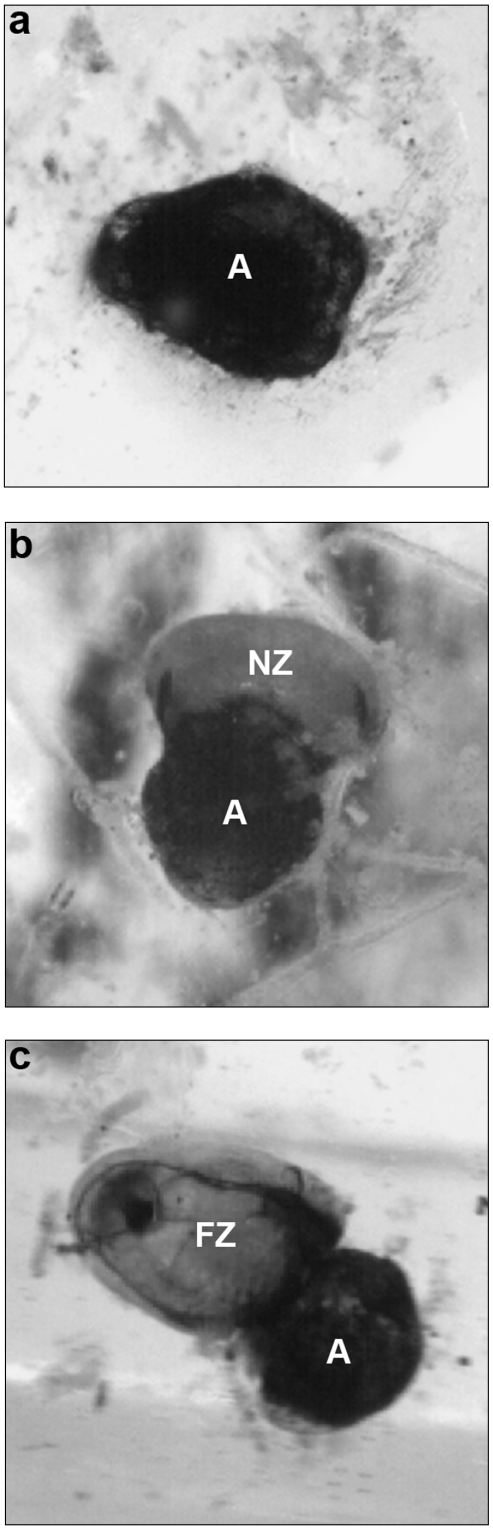

Fig. 6. *Watersipora subtorquata* recruits settled and grown in 100 µg l^{-1} Cu in the laboratory for 20 d then transplanted into the field for 28 d, showing (a) blackened and dead ancestrula (A), (b) formation of a new zooid (NZ) from the blackened ancestrula (A), and (c) fully functional zooid (FZ) budded from the blackened ancestrula (A)

sels can travel to almost any port or harbour in the world within several days or weeks, there exists the enormous potential for nonindigenous species introductions from vessels that are mistakenly considered to be free of and protected from hull-fouling organisms.

Watersipora subtorquata and *Bugula neritina* are cosmopolitan fouling species occurring abundantly in ports and harbours around the globe (Gordon & Mawatari 1992, Hayes et al. 2004). Given that these species may be further exposed to toxicants, such as copper, within the ports and harbours which they inhabit (Paulson et al. 1989, Weis & Weis 1992, 1996, Apte & Day 1998, Hall et al. 1998, Pitt 2002) their tolerance to toxicants may also provide them with the competitive advantage needed to outcompete other organisms within these communities. While pollution may play an important role in mediating the introduction and spread of nonindigenous species such as *Watersipora subtorquata* and *Bugula neritina*, there are undoubtedly other factors that allow toxicant-intolerant nonindigenous marine species (such as *Schizoporella errata* and *Tricellaria occidentalis*) to become successful invasive species. Examples of possible factors include ballast water discharge (Carlton 1989) or physical disturbance (Clark & Johnston 2005). In the context of hull-fouling bryozoan species, the results of this study suggest that *B. neritina* and *W. subtorquata* would be the most common introduced species found in harbours and estuaries.

Bryozoans demonstrated a strong ability to recover from extended periods of Cu exposure. After 26 d in the field, there was no difference in the survival or growth of *Bugula neritina* irrespective of previous Cu exposure. Moreover, regardless of Cu exposure, the average growth rates were similar to those recorded for similarly aged colonies observed in previous studies (Keough 1989).

Following transplantation to the field, recruits of *Watersipora subtorquata* showed no difference in survival across Cu treatments, however colonies exposed to 50 µg l^{-1} Cu had 92% decreased growth after 46 d. Smaller effects on growth have been observed following short term (6 h) exposures of *W. subtorquata* larvae to 100 µg l^{-1} Cu (Ng & Keough 2003). With approximately 200 to 280 zooids per colony after 7 wk, the growth rates of our control colonies were comparable to those of a similar age from other studies (Ng & Keough 2003) suggesting no adverse effects of prolonged laboratory rearing. Given that the survival and growth of just 1 or 2 individuals from a population may be sufficient for a successful invasion to occur, it is worth noting that several recruits of *W. subtorquata* did display remarkable recovery ability in the field following exposure to 100 µg l^{-1} Cu (Fig. 6). All recruits exposed to 100 µg l^{-1} Cu appeared blackened and dead and displayed no feeding activity for the 20 d duration of the laboratory study. After transplantation to the field however, budding and growth was evident from several of the still blackened ancestrula. This remarkable ability of new recruits to 'shut-down' under extended exposure to a toxicant, followed by recovery and growth, gives *W. subtorquata* a potentially enormous competitive advantage over other colonising species; whereby it is able to recruit to highly disturbed areas, lie dormant, and recover once conditions improve.

The ability of these bryozoan colonies to recover following extended periods of Cu exposure stresses the importance for field observations as a component of ecotoxicology studies. This study clearly demonstrates that while larvae and recruits may exhibit adverse effects to toxicants during periods of extended exposure, once this exposure period has passed there may be little or no persistent carry-over effects. This also has important implications for the management and control of invasive marine hull-fouling species. It is not sufficient to simply rely upon relatively short experiments (72 or 96 h) examining larval attachment or metamorphosis to draw conclusions regarding the effectiveness of antifouling strategies. Field transplant observations must follow in order to properly assess the recovery abilities and/or carry-over effects of organisms following periodic toxicant exposure. Such field transplants would also address concerns regarding the real world ecological significance of many ecotoxicology studies (Chapman 2002).

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